5

Thiophanate-methyl, tech. **Oral Developmental Toxicity (83-3a)**

EPA Reviewer: Linnea J. Hansen, Ph.D. Linnea J. Hansen Date 3/22/99
Toxicology Branch I (7509C)
EPA Secondary Reviewer: Susan Makris, M.S. Susan J. Makris, Date 3/23/99
Toxicology Branch I(7509C)

Note: This executive summary is an addendum to a DER dated 5/28/85 (HED Doc. No. 004459) and memorandum dated 2/7/89

DATA EVALUATION RECORD

STUDY TYPE: Developmental Toxicity (Dietary) - Rat [§83-3(a)]

MRID NOS.: 00146643, 92186011

DP BARCODE: D241367
P.C. CODE: 102001

SUBMISSION CODE: S511876
TOX. CHEM. NO.: 375A

TEST MATERIAL (PURITY): Thiophanate-methyl (95.3%)

SYNONYMS: Topsin® M; Dimethyl 4,4'-o-phenylenebis (3-thioallophanate); 1,2-bis(3-methoxycarbonyl-2-thioureido)benzene

CITATION: Keets, S., Leist, P., Mercieca, M (1985) A Dietary Teratology Study of Topsin M Fungicide in Albino Rats: Final Report. WIL Research Laboratories, Ashland, OH. Project No. WIL-75002. Unpublished. (MRIDs 00146643 and 92186011).

SPONSOR: Elf Atochem (Formerly sponsored by Pennwalt Corporation)

EXECUTIVE SUMMARY: In a developmental toxicity study (MRIDs 00146643, 92186011), thiophanate-methyl (95.3% a.i.) was administered in the diet to pregnant Crl:COBS®CD®(SD)BR rats from days 6 through 19 of gestation at concentrations of 0, 250, 1200 or 2500 ppm (equivalent to average daily intakes of approximately 18, 85 or 163 mg/kg/day, respectively).

At 1200 ppm, decreased net body weight gain (-25% less than controls; not significant) and slight but statistically significant decreases in food consumption (g/kg/day) were observed between gestation days 6-9 and 9-12 (-9% and -14% less than controls, respectively). At 2500 ppm, decreased net body weight gain (-36% less than controls; not significant) was observed and consumption was significantly decreased throughout treatment (-15%, gestation days 6-20). There were no treatment-related clinical signs or on cesarean parameters. **The maternal toxicity**

LOAEL is 1200 ppm (85 mg/kg/day), based on marginally decreased body weight gain and food consumption. The NOAEL is 250 ppm (18 mg/kg/day).

No treatment-related developmental toxicity was observed in this study. **The developmental toxicity LOAEL is >2500 ppm (163 mg/kg/day). The NOAEL is ≥2500 ppm.**

This study is classified **Unacceptable (§83-3a)-not upgradable** and does not satisfy the guideline requirement for a developmental toxicity study in the rat. In a memorandum dated 2/7/89 from R. Gardner, TB-I determined that the dietary route of administration was inappropriate for assessment of developmental toxicity of thiophanate-methyl and that a gavage study is required.

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

004459

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Review of a dietary teratology study with TOPSIN® M in rats. EPA Reg. No. 4581-322. Tox. Chem. No. 375A

TO: Phil Hundamann, PM 21
Registration Division (TS-767)

FROM: Roger Gardner, Toxicologist
Section 6
Toxicology Branch Roger Gardner 5-21-85
Hazard Evaluation Division (TS-769)

THRU: Jane Harris, Ph. D., Head JEH 5/21/85
Section 6
Toxicology Branch
Hazard Evaluation Division (TS-769) Chip Walker 5/22/85

Action Requested

Review of a dietary teratology study in rats.

Recommendations and Conclusions

1. Thiophanate methyl, when fed to pregnant rats at levels of 1200 or 2500 ppm on days 6 through 19 of gestation, significantly reduced food consumption. There were no maternal effects, and there were no teratogenic or fetotoxic effects at any of the doses tested.

2. The 250 ppm diet had no maternal or fetal effects and is considered to be the no-observed-effect level.

3. The study is Core Classified as minimum.

I. Background

On December 7, 1977, the Agency issued a Notice of Rebuttable Presumption Against Registration (RPAR) of products containing thiophanate methyl (TOPSIN® M) (FR 42, page 61971). In the Notice, the Agency solicited comments on the potential teratogenic and reproductive effects of the fungicide, and information submitted in response was considered in Position Document 2 (PD2). The Agency concluded:

1 of 29

Tox Chem No. 375A - Thiophanate Methyl File Last Updated 9/13/84

Study/Lab/Study #/Date	Material	EPA Accession No.	Results: LD_{50}, LC_{50}, PIS, NOEL, LEL	TOX Category	CORE Grade/ Doc. No.
Teratology - rat; International Res. and Development Corp.; 1/19/81; exhibit No 47 submitted by Penwalt Corp.	Technical	070997	Preliminary study which found no toxic effects at doses equivalent to 75% of the oral LD_{50}		Invalid 002293
Teratology - rat; International Res. and Development Corp.; 1/29/81; Exhibit No. 48; Penwalt Corp.	Technical	070997	Lower doses in a multigeneration reproduction study suggest that an inappropriate route of administration was used. Low fertility, higher incidence of rudimentary ribs and delayed ossification in control and treated than in historical control animals, reduced litter sizes in all groups below that of historical controls compromises the study.		Invalid 002293 Supplementary 003737
Teratology- mice	TECH (94%)	111934	Teratogenic NOEL > 1000 mg/kg(HDT)		000200
3-Generation reproduction - rat	TECH	111935	Reproductive NOEL = 160 ppm Reproductive LEL = 640 ppm (highest level tested) reduced litter weights, increased pup mortality. Levels tested = 0, 40, 160, 640 ppm		000203
180-Day feeding - rat; Nisso Institute for Life Science	TECH	117761	NOEL = 1600 ppm LEL = 8000 ppm (highest level tested) decreased growth rate, elevated liver weights increased thyroid weight with histologic degeneration. Levels tested = 0, 12.8, 64, 320, 1600, 8000 ppm		000207

APPENDIX II

Data Evaluation Record for Submitted Study

DATA EVALUATION RECORD

1. CHEMICAL: Thiophanate methyl
Dimethyl 4,4'-o-phenylene bis(3-thioallophanate)

2. TEST MATERIAL: TOPSIN® M (Thiophanate Methyl Technical, 95.3% a. i.)

3. STUDY/ACTION TYPE: Teratology - rats

4. STUDY IDENTIFICATION: Rodwell, D. E. April 9, 1985. A dietary Teratology study of TOPSIN® M Fungicide in albino rats: Final Report. Unpublished report no. WIL-75002 prepared by WIL Research Laboratories, Inc., Ashland, OH. Submitted by Pennwalt Corporation, Puyallup, WA. EPA Acc. No. not provided.

5. REVIEWED BY:

Name: Roger Gardner
Title: Toxicologist
Organization: Review Section 6
Toxicology Branch
Signature: Roger Gardner
Date: 5-20-85

6. APPROVED BY:

Name: Jane Harris, Ph. D.
Title: Section Head
Organization: Review Section 6
Toxicology Branch
Signature: _____
Date: _____

7. CONCLUSIONS: Thiophanate methyl, when fed to pregnant rats at levels of 1200 or 2500 ppm on days 6 through 19 of gestation, significantly reduced food consumption. The 250 ppm diet had no maternal or fetal effects.

Core classification: Minimum

> ...significant adverse information on the teratogenic or reproductive effects of thiophanate methyl is not available at the present time.

In response to this conclusion the Registrant submitted two rat teratogenicity studies which were reviewed by the Toxicology Branch (Gardner, 1982; see Appendix I below). The previous review stated:

> An acute LD_{50} of 6,640 mg/kg in female rats...indicates that the dosage range tested (in the preliminary study) is likely to cause mortality in rats. The higher doses are approximately 75, 45, or 15% of the reported LD_{50}, but no mortality or clinical signs of toxicity were reported...
>
> The reduced fertility (in the main study) and the occurrence of hair loss and matted hair coat, along with ambiguous results for fetal effects suggest that the study should be repeated. The need for a new study are underscored by results from a 3-generation reproduction study in which pups were reported to have higher mortality and decreased body weights at birth...The effects were noted at 640 ppm thiophanate methyl in the diet (32 mg/kg/day).
>
> Results from the multigeneration reproduction study further suggest that dietary administration would increase the sensitivity of a teratogenicity study with the fungicide...

The dietary teratology study described herein (see Appendix II below) was submitted in response to the previous review.

Thiophanate methyl is currently being re-evaluated under the Registration Standards program. As part of that process, the 3-generation reproduction study mentioned above has been reviewed again (see Appendix III and Section II. below).

II. Discussion

Pregnant rats were given diets containing 0, 250, 1200, or 2500 ppm thiophanate methyl on days 6 through 19 of gestation. The only effect observed during the study was a decrease in food consumption associated with decreased palatability of the test diets (1200 and 2500 ppm levels). The reduced food consumption was significant during the first few days of treatment when food consumption was decreased as much as 40%. The decreased consumption was also accompanied by decreases

in body weight, but the effects were reversible (see Appendix II).

Consideration of the dietary teratology study along with the multigeneration reproduction study suggest that the reduced viability of pups at birth in the reproduction study may not be related to treatment. Results of the dietary study are also consistent with those of the first teratology study in rats (see Spicer et al, 1981; Appendix III). All of these results suggest that thiophanate methyl is not teratogenic or fetotoxic under the test conditions of the two teratology studies and the multigeneration reproduction study.

III. References

Gardner, R. Memorandum dated November 24, 1982. Subject: Topsin M® (thiophanate methyl). Petition Nos. 0F2420, 1G2463, 2G2662, 2F2729, 2H5342, 2H5364, 2G2639, and 2H5341. 4581-322, and 4581-EUP-35. Tox. Chem. No. 375A. To: Henry Jacoby, Registration Division.

APPENDIX I

Toxicology "One-liners" for Relevant Studies

8. MATERIALS AND METHODS

Test species: Female Crl:COBS® CD® (SD)BR strain rats were used. The animals selected for the study were approximately 13 weeks of age at mating and weighed from 225 to 305 g on the first day of gestation.

Each female was mated overnight with a male, and the following morning each of the females was examined for the presence of a vaginal plug. The day a plug was found was designated Day 0 of gestation.

Experimental procedures: The test substance was mixed with feed and offered to the animals on days 6 through 19 of gestation. Dietary levels of 0, 250, 1200, or 2500 ppm were given to groups of 25 mated dams. Fresh feed was provided weekly during the study.

Test diets were sampled and analyzed for stability of the test substance over a 14-day period under laboratory storage conditions. Samples were also collected for determination of homogeneity of mixtures for each test group.

Each dam was observed twice daily for occurrence of toxic signs and mortality. Body weight determinations were made on days 0 and 6 through 20 of gestation. Food consumption was estimated for gestation days 0 through 6 and daily during the period defined by Days 6 through 20. The report stated that food intake was calculated as g/animal/day and g/kg/day. Compound consumption was also calculated as mg/kg/day.

The rats were sacrificed on day 20 of gestation and subjected to a gross necropsy. Gravid uteri and individual fetuses from each dam were weighed, and the numbers of corpora lutea, implantation sites, live and dead fetuses, and embryonic deaths were noted. Crown-to-rump length was measured for all fetuses.

All fetuses were examined externally. The report stated that approximately half of the fetal heads from each litter were examined for soft tissue effects, and those fetuses were then subjected to visceral examinations. The fetal carcass was then eviscerated and the skeleton was fixed in isopropyl alcohol. The report further noted that the remaining half of the fetuses were examined using the same technique for the viscera, and the brain was examined by a mid-coronal slice. After the skeleton was fixed, the carcasses were cleared with potassium hydroxide, and the skeletons were stained with Alizerin Red S.

8. MATERIALS AND METHODS

Test species: Female Crl:COBS® CD® (SD)BR strain rats were used. The animals selected for the study were approximately 13 weeks of age at mating and weighed from 225 to 305 g on the first day of gestation.

Each female was mated overnight with a male, and the following morning each of the females was examined for the presence of a vaginal plug. The day a plug was found was designated Day 0 of gestation.

Experimental procedures: The test substance was mixed with feed and offered to the animals on days 6 through 19 of gestation. Dietary levels of 0, 250, 1200, or 2500 ppm were given to groups of 25 mated dams. Fresh feed was provided weekly during the study.

Test diets were sampled and analyzed for stability of the test substance over a 14-day period under laboratory storage conditions. Samples were also collected for determination of homogeneity of mixtures for each test group.

Each dam was observed twice daily for occurrence of toxic signs and mortality. Body weight determinations were made on days 0 and 6 through 20 of gestation. Food consumption was estimated for gestation days 0 through 6 and daily during the period defined by Days 6 through 20. The report stated that food intake was calculated as g/animal/day and g/kg/day. Compound consumption was also calculated as mg/kg/day.

The rats were sacrificed on day 20 of gestation and subjected to a gross necropsy. Gravid uteri and individual fetuses from each dam were weighed, and the numbers of corpora lutea, implantation sites, live and dead fetuses, and embryonic deaths were noted. Crown-to-rump length was measured for all fetuses.

All fetuses were examined externally. The report stated that approximately half of the fetal heads from each litter were examined for soft tissue effects, and those fetuses were then subjected to visceral examinations. The fetal carcass was then eviscerated and the skeleton was fixed in isopropyl alcohol. The report further noted that the remaining half of the fetuses were examined using the same technique for the viscera, and the brain was examined by a mid-coronal slice. After the skeleton was fixed, the carcasses were cleared with potassium hydroxide, and the skeletons were stained with Alizerin Red S.

8. MATERIALS AND METHODS (continued)

Uteri found without grossly observable evidence of implantations were placed in ammonium sulfide solution to confirm pregnancy.

9. REPORTED RESULTS

There were no mortalities observed during the study. The most frequently reported clinical sign was alopecia which involved 3 to 6 animals in any group. The investigators noted that the number of animals afftected and the frequency of alopecia was slightly higher in the high dose group than in any other.

According to tabulated data there were 21, 24, 24, and 23 gravid females in the control, low, mid, and high dose groups, respectively. No abortions were observed.

Group mean maternal weight results (g) are summarized as follows:

Dose group	Terminal body wt.*	Net body wt.**	Gravid Uterine wt.	Net body wt. gain**
Control	411	317.2	83.6	60.5
Low	393	327.1	85.5	61.7
Mid	392	311.2	80.7	45.3
High	373	299.2	74.1	39.0

*Includes weight of gravid uterus.
**Excludes weight of gravid uterus.

Reported group mean food consumption values are summarized as follows:

Time (Days of gestation	Dose group Control	Low	Mid	High
	g/animal/day			
0-6	23	22	23	23
6-9	24	22	20**	14**
9-12	25	23	22**	19**
12-15	24	22**	22**	20**
15-20	27	25	25	24**
6-20	25	23**	23**	20**
0-20	24	23*	23*	21**

9. REPORTED RESULTS (continued)

Time (Days of gestation	Control	Dose group Low	Mid	High
	g/kg/day			
0-6	81	81	84	83
6-9	80	76	69**	50**
9-12	80	78	73*	66**
12-15	74	71	70	69*
15-20	72	71	71	70
6-20	76	73	71	65**
0-20	74	73	72	68**

*Significantly different at p=0.05, Dunnet's test
**Significantly different at p=0.01, Dunnet's test

Based on the dietary analyses and food consumption results, the reported group means for consumption of test substance during days 6 through 20 of gestation were 18, 85, and 163 mg/kg/day for the low, mid, and high dose groups, respectively.

There were no significant differences reported between groups with respect to numbers of corpora lutea, implantations, resorptions (early and late), or live fetuses per dam. The group means for these observations are summarized as follows:

Dose group	Corpora lutea	Implantation sites	Total resorptions	Live fetuses
Control	16.7	16.0	1.0	15.0
Low	16.3	15.1	1.2	13.9
Mid	17.3	16.0	0.8	15.2
High	17.3	14.9	0.9	14.0

There were no dead fetuses observed at the time of the laparotomy according to the report.

Mean fetal weights were reported to be 3.6, 3.5, 3.4, and 3.4 g for the control, low, mid, and high dose groups, respectively. Crown to rump lenghts were reported for the respective dose groups to be 3.5, 3.4, 3.5, and 3.5 cm.

The sex ratio (males/females) in the control, low, mid, and high dose groups were 151/163, 158/175, 184/180, and 146/175, respectively.

9. REPORTED RESULTS (continued)

According to tabulated results, there were no external variations observed for any of the fetuses in the study. The most frequently observed soft tissue variations were described as undeveloped renal papillae and/or distended ureters (see Table 1). The most frequently reported skeletal variations included unossified sternebrae (numbers 5 and 6) and 14th rudimentary ribs,

The incidence of fetuses with malformations was not dose-related. The malformations observed in the control group included one fetus with multiple malformations (anasarca, microphthalmia, adactyly, omphalocele, tarsal flexure, and tail absent with anal atresia) and another fetus from another litter with testicular hypoplasia. The low dose group had 3 fetuses in one litter with severe internal hydrocephaly and one fetus in a separate litter with severely malaligned sternebrae. The high dose group had one fetus with situs inversus of the thoracic and abdominal organs.

10. DISCUSSION

Group mean terminal body weight, net body weight (not including gravid uterus weight), net body weight gain (excluding uterine weight), and gravid uterine weight for the high dose group were 9.2, 5.7, 35.6, and 11.4% less than those for the control group, respectively. The mid dose group also showed a decreased body weight gain (25%) in comparison to that for the control group.

Food consumption was comparable for all groups until the test diets were offered from Day 6 of gestation. The overall food consumption from Days 6 through 20 of gestation was decreased in the high dose group by 20%. The largest decrease in food consumption (40% below that for controls) was noted just after dosing began (Days 6-9, see pages 3 and 4 above). These results suggest that the reduced food consumption may be the reason for the reductions observed in terminal body weight, net body weight, net body weight gain, and uterine weight.

The food efficiency, although subject to considerable variability because of food wastage, was evaluated statistically using the t test. Efficiency was determined first by calculation of body weight changes for the control and high dose groups during gestation days 0 to 6, 6 to 20, and 6 to 9.

These calculations were based on individual body weight data

Table 1

Summary of the incidence of most frequently observed variations.

Observation	Control	Low	Mid	High
No. fetuses examined	314	333	324	321
No. litters examined	20	23	24	23
Undeveloped renal papillae and/or distended ureters				
No. of fetuses affected	3	3	9	5
Percentage of fetuses	1.0	0.9	2.5	0.6
No. of litters	2	1	6	3
Percentage of litters	10	4.3	25	13
Unossified sternebrae (#5, #6)				
No. of fetuses affected	39	58	63	48
Percentage of fetuses	12.4	17.4	17.3	15.0
No. of litters	11	12	16	15
Percentage of litters	55.0	52.2	66.7	65.3
14th rudimentary rib				
No. of fetuses affected	17	17	16	12
Percentage of fetuses	5.4	5.4	4.4	3.7
No. of litters	9	11	10	9
Percentage of litters	45.0	47.8	41.7	39.1

10. DISCUSSION (continued)

included in the report. Food consumption for the same periods was also determined from individual animal data with the exception of that for the period represented by days 0 through 6 of gestation. Since an average g food/animal/day was the only reported parameter, the value listed for each animal was multiplied by 6 to get the total food consumption (g/animal). Efficiency was then calculated by dividing the g body weight change by the g food consumed per animal and multiplying the result by 100.

The calculated group mean efficiencies (with standard deviations) for the control and high dose groups are summarized as follows:

Days of gestation	Group mean efficiency (%) Control	High
0-6	19.282 (7.038)	20.535 (5.496)
6-20	27.556 (13.594)	27.731 (10.067)
6-9	15.849 (7.611)	-24.879 (20.420)*

*p<0.001, t test

The negative value for the high dose group mean during days 6 to 9 of gestation reflects the general weight loss (10 g/animal) compared with a general weight gain in the control group (23 g/animal). The group mean food consumptions for that period were 73 and 41 g/animal for the control and high dose groups, respectively.

The lack of a significant difference overall between the control and high dose groups during days 6 through 20 suggests that the reduced body weight, food consumption, and food efficiency may be a result of toxicity and/or palatability. However, toxicity is a more unlikely factor than palatability since the reduced food consumption is consistent with reduced daily intake of the test substance during days 6-9.

Compound consumption was 108, 128, and 140 mg/kg/day for Days 6-7, 7-8, and 8-9, respectively. For the remainder of the dosing period, daily doses ranged from 125 to 193 with an overall average (for the period including days 6-9) of 161.7 (±23.4) mg/kg.

As noted on page 3 above alopecia was the most frequently observed clinical sign. The report stated that it was observed in all groups prior to initiation of the study, and it

10. DISCUSSION (continued)

involved the forepaws, forelegs, and posterior dorsal surfaces of the animals. The report stated:

> Alopecia in the lateral abdominal areas appeared after gestation day 7 in all study groups but in a greater number of animals and with a greater frequency in the 2500 ppm dose group.

As reported, these signs do not appear to clearly coincide with the body weight losses or reduced food consumption described above.

Decreases in gravid uterine weights were not reflected in significantly decreased fetal weights or numbers of live fetuses per litter at the end of the study (see page 4 above). Therefore, uterine weight decreases were not reflective of fetal effects.

In summary, the following factors indicate that the test substance reduced palatability of test diets at 1200 and 2500 ppm.

1. The generally comparable food efficiency during the dosing period for the control and high dose groups along with a significant decrease in food consumption coinciding with the start of test diet administration.

2. The absence of toxic signs coincident with the weight loss and markedly decreased food consumption in the high dose group.

3. The absence of significantly decreased litter sizes or reduced fetal weights in the high dose group which could be associated with the reported decreases in gravid uterine weights.

Although the incidence of unossified sternebrae in fetuses and litters of treated groups shows an increase above that in control group fetuses and litters, the differences are not statistically significant using a 2 X 2 contingency table analysis (Chi square test, $P>0.05$). The incidence of these variations was also within the range in reported historical controls (see Appendix).

There were adequate data presented to indicate that thiophanate methyl, when fed to pregnant rats at levels of 1200 or 2500 ppm on days 6 through 19 of gestation, significantly reduced food consumption. The 250 ppm diet had no maternal or fetal effects.

APPENDIX

Historical Control Data Reported for Skeletal Variations

TOTAL NUMBER OF LITTERS EXAMINED	644
TOTAL NUMBER OF FETUSES EXAMINED EXTERNALLY	9965
TOTAL NUMBER OF FETUSES EXAMINED VISCERALLY	5440
TOTAL NUMBER OF FETUSES EXAMINED SKELETALLY	5931

	NUMBER FETUSES	NUMBER LITTERS	PERCENT (RANGE) FETUSES	PERCENT (RANGE) LITTERS
SKELETAL VARIATIONS				
STERNEBRA(E) #5 AND/OR #6 UNOSSIFIED	1338	422	(4.6- 36.6)	(25.0-100.0)
HYOID UNOSSIFIED	170	104	(0.0- 15.7)	(0.0- 45.0)
14TH RUDIMENTARY RIB(S)	307	165	(0.0- 28.4)	(0.0- 64.0)
STERNEBRA(E) #1,#2,#3 AND/OR #4 UNOSSIFIED	10	35	(0.0- 2.5)	(0.0- 22.7)
REDUCED OSSIFICATION OF THE 13TH RIB(S)	58	43	(0.0- 6.4)	(0.0- 22.7)
STERNEBRA(E) MALALIGNED(SLIGHT OR MODERATE)	50	42	(0.0- 4.3)	(0.0- 29.6)
REDUCED OSSIFICATION OF THE SKULL	44	27	(0.0- 7.7)	(0.0- 19.0)
14TH FULL RIB(S)	6	6	(0.0- 1.4)	(0.0- 7.1)
25 PRESACRAL VERTEBRAE	2	2	(0.0- 0.7)	(0.0- 3.0)
ENTIRE STERNUM UNOSSIFIED	3	3	(0.0- 0.7)	(0.0- 5.0)
METACARPAL(S) AND/OR METATARSAL(S) UNOSSIFIED	4	4	(0.0- 0.9)	(0.0- 9.1)
27 PRESACRAL VERTEBRAE	11	8	(0.0- 2.1)	(0.0- 9.1)
7TH CERVICAL RIB(S)	8	8	(0.0- 1.5)	(0.0- 9.1)
REDUCED OSSIFICATION OF THE VERTEBRAL ARCHES	9	8	(0.0- 1.9)	(0.0- 9.1)
PUBIS UNOSSIFIED	6	6	(0.0- 1.2)	(0.0- 8.3)
BENT RIB(S)	22	14	(0.0- 2.1)	(0.0- 9.5)
GENERAL REDUCED OSSIFICATION OF THE SKELETON	3	3	(0.0- 1.2)	(0.0- 9.0)
REDUCED OSSIFICATION OF THE ISCHIUM	1	1	(0.0- 0.3)	(0.0- 4.5)
REDUCED OSSIFICATION OF THE PELVIC GIRDLE	6	6	(0.0- 1.3)	(0.0- 14.3)
INTERRUPTED OSSIFICATION OF THE 13TH RIB(S)	1	1	(0.0- 0.6)	(0.0- 4.3)

APPENDIX III

Data Evaluation Records for Related Studies

Palmer, A., M. Lovell, and A. Newman. 1972. Effect of Thiophanate Methyl on Reproductive Function of Multiple Generations in the Rat: 4800/72/235. Final Report. (Unpublished report prepared by Huntingdon Research Centre. Submitted by Pennwalt Corporation. Takoma, WA.

Spicer, E., D. Rodwell, C. Graffenius, et al. 1981. Teratology study in rats: 449-006. Unpublished report prepared by International Research and Development Corporation. Submitted by Pennwalt Corp., Agchem Div., Philadelphia, PA.

DATA EVALUATION RECORD

Citation: Spicer, E., D. Rodwell, C. Graffenius, et al. 1981. Teratology study in rats: 449-006. Unpublished report prepared by International Research and Development Corporation. Submitted by Pennwalt Corp., Agchem Div., Philadelphia, PA. MRID No. 106090.

Materials and Methods

Test substance: Thiophanate methyl (purity unspecified, lot no. TM 123) was used.

Test species: Charles River CORS CD-1 strain female rats were used. They were 12 weeks old at mating. Prior to dosing, each female was mated with a male rat, and the day a vaginal plug or sperm were found in a vaginal smear was designated Day 0 of gestation.

Test procedure---Preliminary study: The test substance was dissolved in aqueous gum arabic (5%) and administered to groups of 25 pregnant rats on Days 6 through 19 of gestation. The control group received the vehicle without test substance, and the treated groups were given daily doses of 100, 300, or 1000 mg/kg.

Dams were observed for mortality and changes in appearance and behavior each day. They were observed for signs of toxicity on Days 6 through 20 of gestation; body weights of the dams were measured on Days 0, 6, 9, 12, 16, and 20, and on Day 20 the rats were sacrificed. The abdominal and thoracic cavities, including the organs were examined grossly. Organs with gross lesions were preserved for microscopic examination.

Uteri were removed, and the numbers of corpora lutea, implantations, resorption sites (early and late), as well as live and dead fetuses were noted. Uteri from apparantly nonpregnant animals were placed in 10% ammonium sulfate to confirm pregnancy status.

Fetuses were individually weighed, sexed, and examined externally for abnormalities and variations. Half of them were then prepared for visceral examination, while the remainder was prepared for skeletal observation.

Reported Results

The authors reported no mortalities or compound-related changes in appearance or behavior.

No statistically significant differences (as determined by t-tests) with respect to mean maternal body weights were reported. At Day 20, group mean body weights adjusted by subtracting mean weight of gravid uteri were 338, 336, 335, and 333 g for the control, low, mid, and high dose groups, respectively. The investigators noted that the mean body weight gain during Days 6 through 9 of gestation was less in the treated groups than in the control group (reported group mean weight gains were 9, 7, 7, and 0 g for the control, low, mid, and high dose groups, respectively).

No dams were reported to have totally resorbed litters. One dam in the low dose group delivered on Day 13 of gestation, and one dam in the mid dose group delivered on Day 11.

The authors stated that no statistically or biologically significant differences between historical or concurrent controls and treated groups were found with respect to the number of fetuses or litters with effects. The most frequently noted effects were 14th rudimentary ribs and delayed ossification of sternabrae. Those results are summarized as follows:

	14th rib		Delayed ossification	
Dose group	% fetuses	% litters	% fetuses	% litters
Historical control	10.8	56.1	6.86	48.30
Concurrent control	19.3	55.6	12.2	50.0
Low	12.2	31.6	16.8	36.8
Mid	12.7	58.8	23.7	64.7
High	22.1	62.5	11.7	37.5

One fetus from each of three litters was reported to have malformations. Scoliosis was observed in one, and the second had a bent rib. The third fetus had a small jaw and anasarca according to the report.

Discussion and Conclusions

A low fertility rate was noted by the authors, but they concluded that it did not compromise the results with respect to the fetuses. However, it should be noted that the incidence of fetuses with 14th rudimentary rib is twice that reported historically. The incidence of fetuses with delayed ossification of sternebrae is also two to three times that historically observed in rats at the test facility.

004459

The group mean number of fetuses per litter in the concurrent control and treated groups is lower than that reported for historical controls. The mean litter size for historical controls was reported to be 15, and those reported in the study described here are 12.1, 11.8, 13.9, and 11.1 for the control, low, mid, and high dose groups, respectively.

The authors noted that hair loss from the limbs, as well as the ventral, abdominal, and dorsal posterior surfaces occurred in equal, but unspecified, frequencies for all groups. Animals with matted hair coats were also noted with equal frequency (unspecified) in all groups. Three rats from the control and one in the high dose group were reported to have soft stools at various times during the study also.

The reduced fertility, generally decreased litter size, incidence of hair loss or matting, and ambiguous results with respect to fetal effects suggest that the study should be repeated. However, the need for another study should be reconsidered in view of results from 3-generation reproduction, subchronic feeding, chronic feeding, metabolism, and acute oral toxicity studies.

Core classification: Supplementary for reasons described in the Discussion and Conclusions section above.

DATA EVALUATION RECORD

Citation: Palmer, A., M. Lovell, and A. Newman. 1972. Effect of Thiophanate Methyl on Reproductive Function of Multiple Generations in the Rat: 4800/72/235. Final Report. (Unpublished report prepared by Huntingdon Research Centre. Submitted by Pennwalt Corporation. Takoma, WA. MRID No. 117870.

Materials and Methods

Test substance: Thiophanate methyl was used (no further information was provided).

Test species: Male and female CD strain weanling rats were used. They weighed 70 to 80 g at the beginning of the study.

Experimental procedures: Four groups each containing 10 male and 20 female rats were designated the first parental generation (F_0) in the study. Each group was given diets containing 0, 40, 160, or 640 ppm test substance. Test diets were fed to the F_0 males and females for 60 days prior to mating.

The rats were observed frequently for appearance of toxic signs, changes in behavior, and mortality during the study. The adult animals were weighed once each week from initiation of the study until mating. Females were weighed on Days 0, 7, and 14 during gestation or lactation.

Food consumption was estimated each week from initiation of the study until mating. Thereafter, no food consumption determinations were made according to the report.

One male was cohabited with two females from the same group for up to 20 days to produce F_{1a} offspring. The females of each mating were examined daily for the appearance of a vaginal plug or the presence of sperm in a vaginal smear during the mating period. The day evidence of successful mating was found was designated Day 0 of gestation.

The report stated that 10 days after weaning of the F_{1a} pups the F_0 animals were designated to alternate pairings under the same conditions to produce the F_{1b} offspring.

The day that all pups were delivered was designated Day 0 of lactation. The pups were examined for the appearance of abnormalities, and the numbers of live and dead pups were noted (on Days 0, 4, 12, and 21). On lactation Days 4, 12, and 21 pup weights were determined.

004459

When the F_{1b} pups were weaned 10 males and 20 females were selected to be mated in the next generation (F_2). All F_{1a} pups and F_{1b} pups not chosen to be parental animals were sacrificed and examined for internal and external abnormalities. Those pups found with abnormalities were processed for subsequent microscopic examination.

After selection of the F_1 parental animals, all of the F_0 animals were sacrificed and discarded.

The F_{1b} adults and their offspring (F_{2a} and F_{2b} litters) and the F_{2b} adults and offspring (F_{3a} and F_{3b} litters) were treated in the same manner as the F_0 animals and offspring (F_{1a} and F_{1b} litters) with the exception of the F_{3b} pups. After gross examination of the F_{3b} pups, organs were removed from 10 males and 10 females of each sex in all groups for weighing and microscopic examinations. Those organs included brain, liver, heart, pituitary, spleen, thyroid, kidneys, thymus, adrenals, gonads, and lungs. In addition, tissue samples of the pancreas, bladder, bone, stomach small and large intestine were prepared for histolopathology examination. Femoral bone marrow smears were also prepared for microscopic examination.

Ten additional rats of each sex were taken from each group for skeletal observations. They were cleared and stained with alizarin for those observations.

Reported Results

Parental animals: The investigators noted that there were no treatment-related signs of toxicity observed during the study. There were 9 deaths among female rats, and 2 males rats died during the experiment. The occurrance of deaths in the females is summarized in Table 1.

Table 1

Summary of mortality in pregnant females

	Dose group			
Generation	Control	Low	Mid	High
F_0	0	0	1	1
F_1	2	0	1	0
F_2	1	1	0	2

The differences between reported group mean body weights for the treated and control groups did not exceed 5% during the study for either sex. There was also no significant difference

004459

noted between mean food consumption reported for the control and treated groups. The authors stated, "...there was a tendency for slightly lower weight gain at 160ppm for the F0 and F2B generations and at 640ppm for the F2B generations."

The fertility rates during the first two generations ranged from 65% to 100%, but the authors noted that the mating index (number of confirmed matings/total matings attempted) was generally lower during the second mating of the F_{2b} animals. The mating indices for the first F_{2b} mating ranged from 85 to 95%, and the second mating indices ranged from 35% in the control group to 75% for the mid-dose group.

Total litter losses were reported in 6 dams during the three generations of the study. Two of those were the result of maternal death, and three were reported to exhibit signs of dystocia. The sixth dam had no associated signs. The authors stated that the incidence of total litter loss was low and unrelated to treatment, and on that basis those litters were not considered in the calculations of group means and statistical analyses for litter observations.

Litter observations: The mean number of live pups reported per dam is summarized in Table 2.

Table 2

Summary of the mean number of live pups born per dam for each test group

Generation and mating	Dietary level (ppm) 0	40	160	640
F_{1a}	12.2	11.7	12.7	11.5
F_{1b}	11.7	11.9	10.4	11.4
F_{2a}	12.3	12.1	13.0	10.8
F_{2b}	12.8	12.8	12.7	11.5
F_{3a}	13.1	12.7	12.9	13.3
F_{3b}	12.2	11.1	10.8	10.8

Although no statistically significant decreases in litter sizes at birth, through lactation, and at weaning were noted ($p>0.05$, Wilcoxon test), the authors stated that the high-dose group litter sizes tended to be smaller than those for the control groups throughout the three generations (see Discussion and Conclusions section below).

The authors stated,:

> The only suggestion of a consistent trend over the three generations was the tendency at 640ppm for lower litter weight from birth through lactation to weaning.

The group mean litter weights for the control and high dose groups are summarized in Table 3.

Table 3

Summary of group mean litter weights (g) for the control and high dose groups

Index	Control Matings		High-dose matings	
	First	Second	First	Second
First generation				
Day 0	73.5	73.8	68.4	71.0
Day 4	117.5	118.3	113.1	110.7
Day 21	568.0	554.1	536.9	566.4
Second generation				
Day 0	78.3	83.8	68.7	74.2
Day 4	136.2	139.5	122.2	125.4
Day 21	590.8	671.2	556.0	593.3
Third generation				
Day 0	79.4	78.2	80.7	66.2
Day 4	126.8	124.9	123.3	105.6
Day 21	585.7	595.9	570.3	493.7

This trend was attributed to the lower litter sizes and the tendency of the pups to have lower individual weights (see Discussion and Conclusions section below). None of the differences were described by the authors as statistically significant ($p>0.05$, Wilcoxon test).

For five of the six matings the investigators noted that the incidence of pup mortalities during lactation was slightly lower for the high-dose group than it was in the control group litters. However, they also stated that there was no statistical significance associated with these differences ($p>0.05$, Wilcoxon test; see Discussion and Conclusions section below).

004459

Three pups were reported to have major malformations: one from the first mating in the control group (protrusion of the salivary gland through a mid-line defect in the ventral cervicle region), one in the 40 ppm group (malocclusion of incisors resulting from apparent malalignment of lower jaw), and one from the high-dose group (open eyelids, slight tongue protrusion, and kinked tail; also bipartite/asymmetric lower thoracic centra and absent 5th sternebra). These were not considered to be dose-related.

In the F_{3b} pups organ weights for treated groups were slightly higher than controls with respect to the liver and kidneys. Group mean liver weights were reported to be 510, 566, 521, and 543 mg for the control, low, mid, and high dose groups, respectively. Respective mean kidney weights were reported to be 125, 141, 131, and 133 mg.

No dose-related histopathology was observed in the F_{3b} pups examined according to the report.

Discussion and Conclusions

The differences between group mean body weights for the treated and control parental animals (see Reported Results section, page 2) are unlikely to be toxicologically significant. The fact that these weight differences were not corroberated by a consistent or dose-related decrease in body weight gain (without food consumption decreases) over the three generations of the study substantiates the conclusion. There was also no effect on mortality, fertility, or the number of dams having total litter loss. Based on these considerations a no-observed-effect level (NOEL) for parental toxicity is >640 ppm.

In the absence of statistically significant differences, the reported tendencies toward decreased litter sizes for the high-dose group is of questionable toxicological significance. Gestation, viability, and lactation indices were calculated from individual animal data included in the report, and the results of those calculations are summarized in Table 4.

Those results do not indicate the slight reductions which the investigators described. The reduced viability index observed in the high dose group for the first mating of the third generation was not repeated in the second mating or found in the other two generations. The reduced lactation index for the second set of litters in the third generation was not observed during the first mating or in other generations either.

004459

Three pups were reported to have major malformations: one from the first mating in the control group (protrusion of the salivary gland through a mid-line defect in the ventral cervicle region), one in the 40 ppm group (malocclusion of incisors resulting from apparent malalignment of lower jaw), and one from the high-dose group (open eyelids, slight tongue protrusion, and kinked tail; also bipartite/asymmetric lower thoracic centra and absent 5th sternebra). These were not considered to be dose-related.

In the F_{3b} pups organ weights for treated groups were slightly higher than controls with respect to the liver and kidneys. Group mean liver weights were reported to be 510, 566, 521, and 543 mg for the control, low, mid, and high dose groups, respectively. Respective mean kidney weights were reported to be 125, 141, 131, and 133 mg.

No dose-related histopathology was observed in the F_{3b} pups examined according to the report.

Discussion and Conclusions

The differences between group mean body weights for the treated and control parental animals (see Reported Results section, page 2) are unlikely to be toxicologically significant. The fact that these weight differences were not corroberated by a consistent or dose-related decrease in body weight gain (without food consumption decreases) over the three generations of the study substantiates the conclusion. There was also no effect on mortality, fertility, or the number of dams having total litter loss. Based on these considerations a no-observed-effect level (NOEL) for parental toxicity is >640 ppm.

In the absence of statistically significant differences, the reported tendencies toward decreased litter sizes for the high-dose group is of questionable toxicological significance. Gestation, viability, and lactation indices were calculated from individual animal data included in the report, and the results of those calculations are summarized in Table 4.

Those results do not indicate the slight reductions which the investigators described. The reduced viability index observed in the high dose group for the first mating of the third generation was not repeated in the second mating or found in the other two generations. The reduced lactation index for the second set of litters in the third generation was not observed during the first mating or in other generations either.

Table 4

Gestation[1], viability[3], and lactation[2] indices for the control and high dose groups

Index	Control Matings		High-dose matings	
	First	Second	First	Second
First generation				
Gestation	98	98	98	100
Viability	96	96	95	97
Lactation	99	98	99	99
Second generation				
Gestation	98	99	98	99
Viability	98	99	97	99
Lactation	96	100	99	97
Third generation				
Gestation	100	100	95	97
Viability	96	96	91	97
Lactation	97	100	98	93

[1]Gestation index = $\frac{\text{number of live pups}}{\text{total number of pups born}} \times 100$

[2]Viability index = $\frac{\text{number of pups alive at Day 4}}{\text{number of live pups at birth}} \times 100$

[3]Lactation index = $\frac{\text{number of pups alive at Day 21}}{\text{number of pups alive at Day 4}} \times 100$

Table 3 (see page 4) indicates that in most cases the litter weights for the control and high dose groups at birth, during lactation, and at weaning are slight. The largest group differences appear in both matings of the second generation and in the second mating of the final generation. These differences indicate that the high dose group's mean litter weights for the three matings range from 9 to 17% less than mean litter weights for the controls. The group mean pup weights (g) and litter sizes reported for those matings are summarized in Table 5. A comparison of these data with the appropriate mean litter weights from Table 3 above supports the conclusion of the investigators that the weight differences with respect to the litter results from fewer pups per litter.

Table 5

Summary of group mean pup weights and litter sizes for selected matings

Generation and mating	Dose group: At birth Control	At birth High	At weaning Control	At weaning High
	Pup weight			
F_{2a}	6.4	6.5	51.6	54.3
F_{2b}	6.6	6.5	54.2	55.2
F_{3b}	6.4	6.2	51.3	51.8
	Litter size (live pups)			
F_{2a}	12.3	10.8	11.6	10,4
F_{2b}	12.8	11.5	12.6	10.9
F_{3b}	12.2	10.8	11.7	9.8

A review of the individual animal data for the three matings considered in Table 5 indicated that there were no outlyers with respect to litter size (animals were considered to be "outlyers" if their litters size was two or more standard deviations from the group mean).

These considerations and the absence of dose-related pathology suggest that the effects on the offspring of the high dose group could be associated with the test substance, but this conclusion should be confirmed with results from rats given doses higher than 640 ppm. Such information would also provide a basis for assessing the toxicological significnace of the effects on pups. Because of the uncertainty associated with the results described above a provisional NOEL for effects on offspring is 160 ppm, and the lowest-effect level (LEL) is 640 ppm.

Core classification: Minimum.

END